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ACCUMULATION OF TOXIC ELEMENTS IN FISH FROM OHRID LAKE UNDER THE INFLUENCE OF ANTHROPOGENIC ACTIVITIES

Dijana Blazhekovikj - Dimovska1*, Mirko Ukoski2

1 University "St. Kliment Ohridski", Faculty of Biotechnical Sciences, Partizanska b.b., 7000 Bitola, R. N. Macedonia

2 State Inspectorate for Agriculture, 6330 Struga, R. N. Macedonia

*Corresponding Author: dijana.blazekovic@uklo.edu.mk

INTRODUCTION

According to the definition of the United Nations Environment Agency (UNEP), monitoring is continuous observing, with clearly defined objectives of one or more chemical or biological elements, according to a determined time and space schedule, using comparable and standardized methods. Because the increasing pollution of water ecosystems with various natural and anthropogenic pollutants, including heavy metals, is becoming a great threat to the environment and consumer health, water quality monitoring is an extremely important segment.

Water ecosystems are under intense anthropogenic influence, which leads to an increased presence of pollutants, and a large number of toxic pollutants, primarily heavy metals, which requires permanent monitoring. Heavy metals are chemical elements with a specific gravity that is at least five times greater than the specific gravity of water. Due to their toxicity and ability to bioaccumulate in aquatic biomes, they are among the most serious pollutants in the natural environment (Tam & Wong, 2000; Miller et al., 2002). They tend to accumulate in the aquatic environment because they cannot be degraded, which results in serious problems (Donati, 2018).

In lakes, fish are often at the top of the food chain but also tend to accumulate some heavy metals from the water (Mansour & Sidky, 2002). Therefore, the bioaccumulation of metals in fish can be considered as an index of pollution of aquatic ecosystems with toxic elements (Tawari-Fufeyin & Ekaye, 2007; Karadede-Akin & Unlu, 2007), which is a useful tool for studying the biological role of metals present in fish in higher concentrations (Dural et al., 2007; Anim et al., 2011).

Fish are one of the most indicative factors for assessing water pollution with toxic elements, and this is important not only for environmental protection but also for the assessment of meat quality and the potential risk to the human population. Human exposure to toxic elements primarily comes through the diet, so determining the contamination with these elements in commercially more important fish species is extremely important. On the other hand, such research should analyze more than one fish species in comparative studies of the aquatic environment, due to significant variations in accumulation processes in different fish species (Uysal et al., 2009).

Research objectives in this study were the following: analysis of the level of accumulation of heavy metals and metalloids (arsenic (As), iron (Fe), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) in the muscle tissue of important fish species from Ohrid Lake); comparison of accumulated heavy metals and metalloids in muscle tissue between different fish species; application of the heavy metal pollution index (Metal Pollution Index - MPI) in the assessment of water pollution with heavy metals; and comparison of the concentrations of accumulated heavy metals and metalloids in the muscle tissue of the investigated fish species with the maximum allowed concentrations prescribed by the legal regulations.

MATERIALS AND METHODS

In this paper, the research area was one of the most significant lakes in Macedonia, Ohrid Lake. Mines pollute the environment through mining activities, which accumulates toxic elements on site due to the discharge of untreated wastewater from the extraction and production process. Near Ohrid Lake, on the Albanian side, there are six mines and one mineral enrichment plant, all located at a distance of 2.5 km from the shore of the lake. These mines, as well as four other mines located 10 km from the shore of the lake, are a source of pollution both on the shore and in other parts of the lake. The mines operated at full capacity until the early 1990s, and at the moment only one mine is operating. From here, mining wastewater, essentially untreated, is drained directly to Ohrid Lake. There are large deposits of residual material left in the open pits in the region, which means that every time it rains, meteoric water enters these pits and becomes contaminated, polluting the groundwater and water sources in the surrounding area and eventually ending up in the lake itself. All of these chemical elements originating from the mining industry can reach the lake through rainwater and runoff, through the discharge of surface and groundwater into the lake, and through the air.

For this purpose, the identification of the polluted areas in the littoral zone of Ohrid Lake was carried out. On the Albanian side, the littoral zone near the city of Pogradec is heavily affected (Figure 1). The influences are also visible in the northeast, near Tushemisht, as well as in the western and northwestern parts, Lin and Radozhda, on the Macedonian side. Abandoned mines and their associated waste piles are a source of contamination of water and sediments with toxic elements.

Common carp, Ohrid trout, and barbel are sampled as material for analysis. The research was performed seasonally over one year, with fish samples collected four times for the analysis of heavy metals and metalloids (May, September, December, and February). After catching the fish, the total length (expressed in cm, accuracy \pm 0.1 cm) and mass (expressed in g, accuracy \pm 0.1 g) of the fish were measured and individuals were selected for further analysis according to the commercial size (minimum size of individuals allowed for fishing), based on the Fishing Base for the Ohrid Lake fishing water for 2017 - 2022 (Official Gazette of the Republic of Macedonia No 55/17). Then, each individual was washed with distilled water and dissected in a fresh state.

Fig 1. Fishing sites and locations of potential pollutants (1 former iron and chrome factory; 2 - tailings; 3 – fishing site)

Dorsal muscle tissue from the right side of the dorsal fin (t) 5 g) was separated with a sterilized knife and transported to the laboratory on ice in separate plastic "zipper" bags in a portable hand-held refrigerator where the samples were frozen and stored at -20 °C until further analyses.

An Atomic Absorption Spectrophotometer, type Agilent Technologies 200 Series AA Systems (GTA 120 Graphite Tube Atomizer) was used for the analysis of heavy metals and metalloids.

The following formula was used to calculate the Metal Pollution Index (MPI):

$$
MPI = (cf_1 \times cf_2 \times cf_3 \times ... cf_n)^{1/n}
$$
 (Usero et al., 1997),

where cf_n is the concentration of the metal in n f^th sample. Standard statistical methods (Microsoft Office Excel 2010, Data Analysis ToolPak) were used for the result processing. To assess the differences in the levels of toxic elements between the fish species studied, a t-test analysis was conducted to determine whether there were significant variations in the concentrations of toxic elements between these species.

RESULTS AND DISCUSSION

The results for the concentrations (mg/kg) of toxic elements in the three fish species (common carp, Ohrid trout, and barbel) by sampling months are shown in Tables 1 - 4. The values are shown as mean values and standard deviation ($\bar{x} \pm SD$), as well as the concentration range (minimum and maximum value).

In the research done in May (Table 1), when determining iron (Fe), the lowest mean value was determined in trout (0.00399 mg/kg), while the highest mean value was found in carp (0.01023 mg/kg). Regarding chromium (Cr), the lowest mean value was also determined in trout (0.44359 mg/kg), and the highest in carp (0.66333 mg/kg). The highest mean value of copper (Cu) was observed in barbel (0.112 mg/kg) , and the lowest in carp (0.06434 mg/kg) . Regarding the concentration of lead (Pb), the highest mean value was determined in barbel (0.08767 mg/kg), and the lowest in trout (0.0671 mg/kg). The highest mean value of zinc (Zn) was determined in barbel (9.858 mg/ kg), and the lowest in trout (8.124 mg/kg).

In the research conducted in September (Table 2), when determining iron (Fe), the lowest mean value was determined in trout (0.0041 mg/kg), and the highest in barbel (0.102 mg/kg). Regarding chromium (Cr), the lowest mean value was also determined in trout (0.49868 mg/kg), and the highest in barbel (0.67283 mg/ kg). The highest mean value of copper (Cu) was in trout (0.10615 mg/kg), and the lowest in barbel (0.07822 mg/ kg). Regarding the concentration of lead (Pb), the highest mean value was determined for barbel (0.08356 mg/kg), and the lowest for carp (0.08143 mg/kg). The highest mean value of zinc (Zn) was determined in barbel (9,766 mg/kg), and the lowest in trout (8,092 mg/kg).

In the research conducted in December (Table 3), when determining iron (Fe), the lowest mean value was determined in trout (0.0042 mg/kg), and the highest mean value in barbel (0.102 mg/kg). Regarding chromium (Cr), the lowest mean value was also determined in trout (0.49442 mg/kg), and the highest in barbel (0.67123 mg/ kg). The highest mean value of copper (Cu) was observed in barbel (0.10771 mg/kg), and the lowest in carp (0.10446 mg/kg). Regarding the concentration of lead (Pb), the highest mean value was determined for carp (0.08655 mg/kg), and the lowest for barbel (0.08261 mg/kg). The highest mean value of zinc (Zn) was determined in barbel (9.651 mg/kg), and the lowest in trout (8.022 mg/kg).

In the research carried out in February (Table 4), when determining iron (Fe), the lowest mean value was determined in trout (0.00381 mg/kg), and the highest mean value in barbel (0.0983 mg/kg). Regarding chromium (Cr), the lowest mean value was determined in trout (0.49351 mg/kg), and the highest in barbel (0.67132 mg/kg).

Table 1. Concentration of toxic elements in common carp, Ohrid trout and barbel in May (\bar{x} ± SD) (min - max)

Table 2. Concentration of toxic elements in common carp, Ohrid trout and barbel in September (\bar{x} ± SD) (min - max)

Table 3. Concentration of toxic elements in common carp, Ohrid trout and barbel in December (\bar{x} ± SD) (min - max)

Table 4. Concentration of toxic elements in common carp, Ohrid trout and barbel in February (\bar{x} ± SD) (min - max)

© 2024 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/) The highest mean value of copper (Cu) was observed in barbel (0.1077 mg/kg), and the lowest in carp (0.10446 mg/kg). Regarding the concentration of lead (Pb), the highest mean value was determined in carp (0.08655 mg/ kg), and the lowest in trout (0.0825 mg/kg). The highest mean value of zinc (Zn) was observed in barbel (9.669 mg/kg), and the lowest in trout (8.014 mg/kg).

Concentrations of toxic elements in the muscle tissue of freshwater fish vary considerably between different studies (Chattopadhyay et al., 2002; Papagiannis et al., 2004), probably due to differences in metal concentrations and chemical characteristics of the water from which the fish were sampled, environmental conditions, the metabolism, and nutrition of the fish, as well as the season in which the research was carried out. In general, heavy metals are naturally found in very low concentrations, while increased concentrations are usually associated with pollution caused by human activities (Idodo-Umeh, 2002).

Different fish species exhibit variations in their feeding habits, metabolism, and physiological characteristics, which can affect the uptake and accumulation of toxic elements. Environmental factors such as water temperature, rainfall, and water chemistry can vary in different months of the year. These seasonal variations can affect the availability and bioavailability of toxic elements in the aquatic ecosystem. Therefore, significant differences can be observed in the levels of toxic elements in fish samples collected in different months (Magu et. al., 2016; Naeem, 2021).

The interaction between fish species and season may play a role in the accumulation of toxic elements in fish. Some species may exhibit stronger seasonal variations in element levels than others. For example, certain fish species may have greater accumulation of toxic elements during the warmer months of the year due to increased metabolic activity, while other species may show different patterns of element accumulation across seasons, depending on the specific toxic elements being analyzed, the geographic location and the characteristics of the fish species being studied (Monier et al., 2023).

To determine whether the meat of the studied fish species is safe for human consumption, the concentrations of toxic elements in fish muscle tissue were compared with the maximum permissible concentrations (MPC) in fish meat for human consumption established by the European Union (EU) and national legislation. According to EU regulations (Regulation of the European Commission, 2006), the MPCs for Cd and Pb are 0.05 and 0.30 mg/kg per unit of fresh mass, the MPLs for As, Cd and Pb in fish meat are 2.0, 0.1 and 1.0 mg/kg, while the MPLs for Zn, Cu and Fe (for fish products in tin packaging) are 100.0, 30.0 and 30.0 mg/kg per unit of fresh mass, respectively. Based on the data in Tables 1 - 4 which show the concentrations of toxic elements (Fe, Cr, Cu, Pb, Zn) in the studied fish species (common carp - C, Ohrid trout - T, barbel - B) during different months (May, September,

December, February), the values are below the maximum permitted concentrations (MDC) prescribed based on the Regulation of the European Commission (EU) (1881/2006). The differences in the levels of toxic elements between the studied fish species, investigated using t-test analysis are shown in Table 5.

There was a significant difference (*P* < 0.05) in iron levels between fish species in May. Barbel had the highest average iron concentration (0.010 mg/kg), while trout had the lowest (0.003 mg/kg). Similar trends can be observed in September, December, and February, indicating persistent differences in iron accumulation between fish species. Iron concentrations were significantly higher during the warmer months, compared to other months. This knowledge stems from the increased biological activity and metabolic processes of fish during the warm months of the year, which leads to the increased intake of iron by fish. T-test analysis indicates that there are significant differences ($P < 0.05$) in iron levels in fish in different months.

Chromium concentrations showed variation between fish species in different months. Average chromium concentrations were different for each fish species in May, September, December, and February. For example, in May barbel had the highest average chromium concentration (0.613 mg/kg) , while trout had the lowest (0.443 mg/s) kg). This difference is statistically significant (*P* < 0.05), indicating that chromium levels in different fish species are not similar. In September, December, and February, barbel consistently showed higher levels of chromium than other species, while trout generally had the lowest levels of chromium. These findings suggest that chromium accumulation in fish is influenced by species-specific factors such as feeding habits, habitat preferences, or metabolic processes.

There was a significant difference (*P* < 0.05) in copper levels between fish species in May. Barbel had the highest average concentration of copper (0.112 mg/kg), while carp had the lowest (0.064 mg/kg). This significant difference in copper levels between species was also observed in September, December, and February, indicating consistent variations in copper accumulation between different species. Comparing these concentrations with the MPC of 10 mg/kg for copper, it can be noted that all recorded concentrations of copper in fish species were far below the permissible limit. This indicates that the fish samples analyzed in these surveys comply with regulatory guidelines regarding allowable levels of copper. Our research is consistent with studies that have reported variations in copper accumulation between fish species due to species-specific physiological differences and feeding habits (Chen et al., 2018).

Comparing the obtained values with the MPC of 0.3 mg/ kg for lead, it can be noted that the concentration of lead in the investigated fish species in all months is below the permissible limit.

Table 5. Significance of the t-test values for the presence of toxic elements (Fe, Cr, Cu, Pb and Zn) in the muscle tissue of different fish species from Ohrid Lake, by months

* C- common carp; T- Ohrid trout; B- barbel

a,b,c The differences in the values shown with the same superscripts per column, for each element separately and in different fish species, are statistically significant at a significance level of *P* < 0.05

Lead is a toxic element that can have harmful effects on human health, especially when consumed above the permissible limits for a long period. It is important to investigate the potential sources of lead contamination in the aquatic environment and to determine measures to mitigate its presence.

There was a significant difference $(P < 0.05)$ in zinc levels between different species in May. Barbel had the highest average concentration of zinc (9.858 mg/kg), while trout had the lowest (8.124 mg/kg). Similar results were observed in September, December, and February, indicating consistent differences in zinc accumulation between fish species. The maximum permissible concentration for zinc is 50 mg/kg. Based on the obtained results, the measured concentrations of zinc in sampled fish species during the examined months are significantly below the MPC. This suggests that the fish species analyzed do not pose a risk to consumer health in terms of zinc contamination.

Arsenic is a metalloid that is often present in the environment in higher concentrations, and it is toxic to plants, animals, and humans, but its toxicity varies as a result of different chemical forms. It is widely distributed, occurring in soil, water, and air. According to research by Has-Schon et al. (2006), Višnjić-Jeftić et al. (2010) and Squadrone et al. (2013), the concentration of arsenic is low in most fish, but the highest concentration is always found in muscle tissue. Arsenic was not detected in any investigated fish species in our study.

CONCLUSION

The interpretations of these results are based on the data obtained from our research, and a comprehensive assessment would require multi-year research and monitoring. Any differences in the obtained values for the concentrations of the examined toxic elements may be influenced by the type of diet specific to fish species, their habitat, environmental factors such as water chemistry and sediment composition, as well as metal uptake mechanisms in different fish species. The results obtained provide specific knowledge about the accumulation of the examined toxic elements in certain fish species from Ohrid Lake and can help in the further planning of environmental monitoring and conservation efforts, as well as in developing awareness of the risks of pollution with these elements and their impact on human health. In this way, it will be possible to establish practical and effective measures for this environmental and public health problem.

AKUMULACIJA OTROVNIH ELEMENATA U RIBA-MA IZ OHRIDSKOG JEZERA POD UTJECAJEM ANTROPOGENIH AKTIVNOSTI

SAŽETAK

Vodeni ekosustavi su pod intenzivnim antropogenim utjecajem, što dovodi do povećane prisutnosti onečišćujućih tvari, uključujući veliki broj toksičnih tvari, prvenstveno teških metala, što zahtijeva stalni nadzor.

Riba je jedan od najindikativnijih čimbenika za procjenu onečišćenja voda štetnim elementima, a to je važno ne samo za zaštitu okoliša, nego i za procjenu kakvoće mesa i potencijalnog rizika za ljudsku populaciju. Glavni cilj ovog istraživanja bio je analizirati razinu akumulacije teških metala i metaloida, kao što su arsen (As), željezo (Fe), krom (Cr), bakar (Cu), olovo (Pb) i cink (Zn) u mišićnom tkivu komercijalno važnih vrsta riba iz Ohridskog jezera, usporediti njihove koncentracije u različitim vrstama riba, primijeniti indeks onečišćenja teškim metalima u procjeni onečišćenja vode, te napraviti usporedbu koncentracije akumuliranih teških metala i metaloida u mišićnom tkivu ispitivanih vrsta riba s maksimalno dopuštenim koncentracijama propisanim zakonskim propisima. Područje istraživanja bilo je Ohridsko jezero, Makedonija. Za analizu su uzorkovane najvažnije komercijalne vrste riba iz Ohridskog jezera, odnosno šaran, ohridska pastrva i mrena. Statistička analiza podataka pokazala je značajne razlike (*P* < 0,05) i značajne varijacije u razinama željeza, kroma, bakra i cinka između različitih vrsta riba i mjeseci uzorkovanja, naglašavajući važnost razmatranja čimbenika specifičnih za vrstu kao što su hranjenje, preferencije staništa, i metaboličkih procesa u razumijevanju obrazaca nakupljanja elemenata, ali i vremenske dinamike nakupljanja elemenata u ribama. Na temelju podataka o koncentracijama toksičnih elemenata (Fe, Cr, Cu, Pb, Zn) u šarana, ohridske pastrve i mrene tijekom ispitivanih mjeseci (svibanj, rujan, prosinac, veljača) može se zaključiti da su dobivene vrijednosti ispod su maksimalno dopuštenih koncentracija propisanih temeljem Uredbe Komisije (EU) br. 1881/2006. To znači da su analizirane vrste ribe sigurne za konzumaciju i u skladu s regulatornim smjernicama, čime se osigurava zaštita zdravlja potrošača.

Ključne riječi: toksični elementi, šaran, ohridska pastrva, mrena, Ohridsko jezero

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